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DATA EVALUATION RECORD

STUDY TYPE: Developmental Toxicity Study in Rabbits
OPPTS Number: 870.3700 OPP Guideline Number: §83-3b

DP BARCODE: D246707 SUBMISSION CODE: S543930
P.C. CODE: 112600 TOX. CHEM. NO.: None

TEST MATERIAL (PURITY): Prohexadione calcium (91.9% a.i.)

SYNONYMS: Cyclohexanecarboxylic acid; calcium salt of 3,5-dioxo-4-propionyl-cyclohexane-1-carboxylic acid; BX-112

CITATION(s): Kawanishi, Hiroaki. (1992) BX-112: Teratology Study in the Rabbits. Toxicology Research Center, Imamichi Institute for Animal Reproduction, Ibaraki, Japan. Laboratory Project ID. 275, July 7, 1992. MRID 44457762. Unpublished

SPONSOR: BASF Corporation, Agricultural Products, P.O. Box 13528, Research Triangle Park, North Carolina

EXECUTIVE SUMMARY: In a developmental toxicity study (MRID 44457762), prohexadione calcium (BX-112; 91.9% a.i.) was administered by gavage at 0, 30, 100, or 350 mg/kg/day to pregnant New Zealand White SPF rabbits (18 females/dose) on gestation days (GDs) 6 through 18. Does were sacrificed on GD 28. No deaths occurred over the treatment interval. When compared to concurrent controls, no treatment-related clinical signs of toxicity or differences in body weights, body weight gains, food consumption, or gross pathological findings were noted at any dose level tested.

One control doe died on GD 22. At the high-dose, two premature deliveries occurred on GDs 24 and 26 and decreases in the numbers of corpora lutea/dam (↓9%) and implantations/dam (↓14%) were observed (p=not statistically significant). Increases (p=not statistically significant) were noted in the mean preimplantation loss in the low- (↑163%), mid- (↑180%), and high-dose (↑180%) levels; however, these increases were not dose-dependent. The number of resorptions/dam, postimplantation losses, percent male, and fetal weights were similar between control and treated groups.

The maternal LOAEL is 350 mg/kg/day, based on premature deliveries. The maternal NOAEL is 100 mg/kg/day.

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There were no treatment-related effects on developmental parameters noted at any dose level.

The developmental LOAEL was not observed. The developmental NOAEL is ≥350 mg/kg/day.

This developmental toxicity study is classified **acceptable (§83-3[b])** and does satisfy the guideline requirement for a developmental toxicity study in the rabbit.

COMPLIANCE: Signed and dated GLP, Quality Assurance, Data Confidentiality, and Flagging statements were provided.

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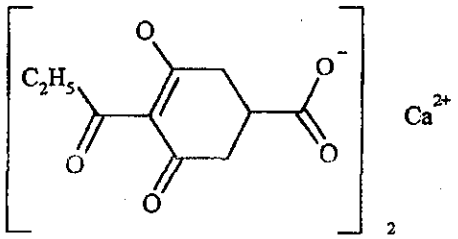
I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Prohexadione calcium; BX-112
   Description: Yellow brown powder
   Lot/Batch #: G14-13
   Purity: 91.9% a.i.
   Storage: Cold dark room (4°C)
   CAS #: 127277-53-6
   Structure:



2. Vehicle: 0.5% carboxymethylcellulose

3. Test animals: Species: Rabbit
   Strain: New Zealand White SPF
   Age and weight of females at mating: 5-6 months old, 2.52-3.92 kg
   Source: Ichikawaya Co. Ltd, Tokyo, Japan
   Housing: Individually in aluminum cages
   Diet: Solid feed for rabbits RM-3 (Funabashi Nojyo Co. Ltd, Chiba, Japan), ad libitum
   Water: Tap water, ad libitum
   Environmental conditions:
   Temperature: 18-25°C
   Humidity: 40-70%
   Air changes: 5-10/hour
   Photoperiod: 14 hour lighting
   Acclimation period: Not reported

B. PROCEDURES AND STUDY DESIGN

1. In life dates - start: 3/28/91 end: 5/23/91

2. Mating: One male was housed with one female and the next day of copulation was designated as gestation day (GD) 0.

3. Animal assignment: Animals were assigned to dose groups based on GD 0 body weights as indicated in Table 1.

Table 1. Animal assignment

Test Group	Dose (mg/kg/day)	Concentration (mg/mL)	Number of Females
Control	0	0	18
Low	30	3	18
Mid	100	10	18
High	350	35	18

4. Dose selection rationale: Doses were based on the results of a pilot study in which pregnant New Zealand White rabbits (5/dose) were administered prohexadione calcium by gavage at dose levels of 0, 20, 100, 250, 500, or 1000 mg/kg/day on GDs 6-18. Does were sacrificed on GD 28. One 1000 mg/kg doe died on GD 15; a 500 mg/kg dam was found dead on GD 11, the result of a gavage error. No treatment-related changes in clinical signs or body weights were observed; reduced (p=not statistically significant) body weight gains were noted at 250 mg/kg (↓57%) on GDs 12-15, however, no dose-dependent relationship was observed at this interval. On GDs 18-28, decreased (p=not statistically significant) body weight gains were noted at the 500 mg/kg (↓100-150%) and 1000 mg/kg (↓44-125%) levels. Decreased (p=not statistically significant) food consumption was observed at 250 mg/kg (↓14-28%) on GDs 6-9; reduced (p=not statistically significant) food consumption was also noted at 500 mg/kg (↓19-60%) and 1000 mg/kg (↓24-85%) on GDs 6-10. No treatment-related gross pathological or reproductive changes were noted at any dose level tested. No fetal examination information was provided.

   Based on the results of this range finding study, the doses presented in Table 1 were selected for the subsequent full developmental toxicity study.

5. Dosage preparation and analysis - Dose solutions were prepared weekly by mixing the appropriate amount of test substance with 0.5% carboxymethylcellulose; solutions were stored in the dark at 4°C. Prior to the study, dose formulations of 3, 10, and 35 mg/mL were evaluated for homogeneity (top, middle, bottom). For stability analyses, samples of the 3 and 35 mg/mL formulations were stored in the dark at 4° C for up to 7 days. Concentration analyses of the three formulations were performed three times during the study.

   Results - Homogeneity analysis (% of nominal±S.D.): low-dose, 93±0.05%; mid-dose, 97±0.17%; high-dose, 99±1.05%.

   Stability analysis: Dose formulations (mean % of nominal) were stable at 4° C for up to 7 days (95% and 107%).

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Concentration analysis (mean % of nominal±S.D.): 3 mg/mL, 93±0.01%; 10 mg/mL, 98%±0.02; 35 mg/mL, 99±0.11%.

The analytical data indicated that the mixing procedure was adequate and that the variance between nominal and actual dosage to the study animals was acceptable.

6. Dosage administration: All doses were administered once daily by gavage on GDs 6 through 18 in a volume of 10 mL/kg body weight. Dosing was based on GD 6 body weights. Control animals received the vehicle, 0.5% carboxymethylcellulose, only.

C. OBSERVATIONS

1. Maternal observations and evaluations - The animals were checked for mortality and clinical signs of toxicity at least once daily throughout the study and twice daily during the treatment interval. Body weight data were recorded on GDs 0, 3, 6, 9, 12, 15, 18, 23, and 28. Food consumption (g/animal/day) was measured daily. Does were sacrificed by air embolus on GD 28. Examinations at sacrifice consisted of a gross exam of the thoracic and abdominal cavities. The reproductive tract was removed, examined, and the following were recorded:
   - pregnancy status
   - number of corpora lutea in each ovary
   - number of implantation sites
   - number and distribution of fetuses (live and dead)
   - number and distribution of resorptions (early and late)

2. Fetal evaluations - All fetuses were weighed, sexed, examined for external abnormalities, and subjected to a visceral examination according to STUCKHARDT's technique. All fetuses were fixed in alcohol and cleared according to DAWSON's method to allow for skeletal examination.

D. DATA ANALYSIS

1. Statistical analyses: All data collected were subjected to routine appropriate statistical procedures.

2. Indices: The following indices were calculated by the investigator:

   Implantation rate: # implantations/# corpora lutea x 100

   Fetal mortality: # fetal deaths/# of implantations x 100

   Sex ratio: # of live male fetuses/# of live female fetuses x 100

3. Historical control data: No historical control data were provided.

significant, and the standard deviations were large.

4. Gross pathology - When compared to concurrent controls, no treatment-related gross pathological findings were noted at any dose level.

5. Cesarean section data - Cesarean section observations are presented in Table 3. One control doe died on GD 22 and two premature deliveries occurred at the high-dose on GDs 24 and 26; premature deliveries were not included in fetal abnormality examinations. Treatment-related decreases (p=not statistically significant) in the numbers of corpora lutea/dam (↓9%) and implantations/dam (↓14%) were observed at the high-dose level. In addition, increases (p=not statistically significant) were noted in the mean preimplantation loss percents in the low- (↑163%), mid- (↑180%), and high-dose (↑180%) levels; however, these increases were not dose-dependent. The number of resorptions/dam, postimplantation losses, percent male, and fetal weights were similar between control and treated groups.

Table 3. Cesarean section observations [a]

Observation	Dose (mg/kg/day)			
	0	30	100	350
# Animals Assigned (Mated)	18	18	18	18
# Animals Pregnant Pregnancy Rate (%)	18 (100)	18 (100)	17 (94)	18 (100)
# Nonpregnant	0	0	1	0
# Total Females Died # Died Pregnant # Died Nonpregnant # Aborted (Partial or complete abortion) # Premature deliveries	1 1 0 0 0	0 0 0 0 0	0 0 0 0 0	0 0 0 0 2
Total # Corpora Lutea Corpora Lutea/Doe	166 9.8±2.1	178 9.9±1.7	166 9.8±2.5	143 8.9±1.6
Total # Implantations Implantations/Doe	161 9.5±2.0	164 9.1±1.7	152 8.9±2.3	131 8.2±1.9
Does with Viable Fetuses	16	18	17	16
Total # Live Fetuses Live Fetuses/Doe	141 8.3±3.1	158 8.8±1.4	146 8.6±2.5	126 7.9±1.8
Total # Dead Fetuses Dead Fetuses/Doe	0 0	0 0	0 0	0 0
Total # Resorptions Early Late Resorptions/Doe Early [b] Late [b] Litters with Total Resorptions	20 10 10 1.2±2.5 0.63 0.63 1	6 0 6 0.3±0.8 0 0.33 0	6 3 3 0.4±0.6 0.18 0.18 0	5 3 2 0.3±0.5 0.19 0.13 0
Mean Fetal Weight (g) Males Females	NR 40.9±6.6 39.1±7.4	NR 41.5±4.6 40.0±6.1	NR 39.8±7.8 40.5±7.4	NR 39.7±5.1 38.4±5.3
Sex Ratio (% Male) [b]	52.5	53.8	45.2	61.1
Preimplantation Loss (%) [b]	3.0	7.9	8.4	8.4
Postimplantation Loss (%) [b]	12.4	3.6	3.9	3.8

a Data extracted from the study report, Table 12, page 37, Appendices 8-1 through 8-4, pages 80 through 83, and Appendices 12-1 through 12-4, pages 95 through 99.
b Calculated by reviewers.
Preimplantation loss = # corpora lutea - # implantations/# corpora lutea
Postimplantation loss = # implantations - # live fetuses/# implantations

B. DEVELOPMENTAL TOXICITY: Fetal examinations included external, visceral, and skeletal observations at necropsy.

1. External examination - There were no treatment-related external malformations or

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variations detected at any dose level. The most common observations are shown in Table 4a. All of the observations at 30 mg/kg were found in a single fetus.

Table 4a. External examinations [a, b]

Observations	Dose (mg/kg/day)			
	0	30	100	350
#Fetuses (#litters) examined	141 (16)	158 (18)	146 (17)	126 (16)
Malformations				
Acephaly	0 (0)	0.6 (5.6)	0.7 (5.9)	0 (0)
Rachischisis	0 (0)	0.6 (5.6)	0 (0)	0 (0)
Ectrodactyly	0 (0)	0.6 (5.6)	0 (0)	0 (0)
Syndactyly	0 (0)	0.6 (5.6)	0 (0)	0 (0)
Meningocele	0 (0)	0 (0)	1.4 (11.8)	0 (0)
Gastroschisis	0 (0)	0 (0)	1.4 (11.8)	0 (0)

a Data extracted from the study report, page 17 and Tables 12 and 13, pages 37 and 38.
b For individual observations, data are presented as % fetal incidence (% litter incidence). Both were calculated by reviewers.

2. Visceral examination - There were no visceral malformations or variations observed at any dose level.

3. Skeletal examination - There were no treatment-related skeletal malformations or variations observed at any dose level. The most common skeletal findings are presented in Table 4b. The variation, splitting vertebrae, was observed in 3 fetuses from 2 litters.

Table 4b. Skeletal examinations [a, b]

Observation	Dose (mg/kg/day)			
	0	30	100	350
#Fetuses (#litters) examined	141 (16)	158 (18)	146 (17)	126 (16)
Malformations				
Skull				
Malformed	0.7 (6.3)	0 (0)	0 (0)	0 (0)
Absent except a part of mandible	0 (0)	0.6 (5.6)	0 (0)	0 (0)
Parietal, hypoplasia	0 (0)	0 (0)	1.4 (11.8)	0 (0)
Absent except a part of occipital	0 (0)	0 (0)	1.4 (11.8)	0 (0)
Sternebrae				
Fusion and/or splitting	1.4 (12.5)	0.6 (5.6)	0 (0)	0 (0)
Vertebrae				
Caudal vertebrae, splitting	1.4 (12.5)	0 (0)	0 (0)	0 (0)
Lumbar vertebrae, disorder	1.4 (12.5)	0 (0)	0 (0)	0 (0)
Cervical vertebrae arches, disruption	0 (0)	0.6 (5.6)	0 (0)	0 (0)
Caudal vertebrae, fusion	0 (0)	0 (0)	0.7 (5.9)	0 (0)
28 pre-sacral vertebrae	0 (0)	0 (0)	0 (0)	0.8 (6.3)
Forelimbs				
Metacarpals, absent and hypoplasia	0 (0)	0.6 (5.6)	0 (0)	0 (0)
Manusphalanges, absent	0 (0)	0.6 (5.6)	0 (0)	0 (0)
Hindlimbs				
Pediphalanges, absent and hypoplasia	0 (0)	0.6 (5.6)	0 (0)	0 (0)
Variations				
Splitting vertebrae	0.7 (6.3)	0.6 (5.6)	0.7 (5.9)	2.4 (12.5)

a Data extracted from the study report, Table 13, page 38.
b For individual observations, data are presented as % fetal incidence (% litter incidence). Both were calculated by reviewers.

III. DISCUSSION

A. INVESTIGATORS' CONCLUSIONS - Administration of prohexadione calcium resulted in maternal toxicity manifested by decreased body weight gains and premature delivery in 2/18 high-dose dams. The maternal NOAEL is 100 mg/kg.

There were no treatment-related external, visceral, or skeletal malformations or variations in this study. The developmental NOAEL is 350 mg/kg/day.

B. REVIEWER'S DISCUSSION

1. MATERNAL TOXICITY: Prohexadione calcium (BX-112; 91.9% a.i.) was administered by gavage at 0, 30, 100, or 350 mg/kg/day to pregnant New Zealand White SPF rabbits (18 females/dose) on GDs 6-18. The analytical data indicated that the mixing procedure was adequate and that the variance between nominal and actual dosage to the study animals was acceptable. Does were sacrificed on GD 28. No treatment-related deaths occurred over the treatment interval; one control doe died on GD 22, the result of a gavage error. When compared to concurrent controls, no treatment-related clinical signs of toxicity or differences in body weights, body weight gains, food consumption, or gross pathological findings were noted at any dose level tested.

   One control doe died on GD 22 and two premature deliveries occurred at the high-dose level on GDs 24 and 26. Decreases (p=not statistically significant) in the numbers of corpora lutea/dam (↓9%) and implantations/dam (↓14%) were observed at the high-dose level. Increases (p=not statistically significant) were noted in the mean preimplantation loss percents in the low- (↑163%), mid- (↑180%), and high-dose (↑180%) levels; however, these increases were not dose-dependent. The number of resorptions/dam, postimplantation losses, percent male, and fetal weights were similar between control and treated groups.

   Maternal LOAEL = 350 mg/kg/day, based on premature deliveries
   Maternal NOAEL = 100 mg/kg/day

2. DEVELOPMENTAL TOXICITY: There were no treatment-related developmental effects noted at any dose level.

   a. Deaths/Resorptions: The numbers of resorptions/doe and viable fetuses/doe for the treatment groups were not significantly different from the concurrent controls.
   b. Altered Growth: There were no treatment-related changes in fetal body weights at any dose level.
   c. Developmental Variations: There were no treatment-related developmental variations noted at any dose level.
   d. Malformations: There were no treatment-related developmental malformations noted at any dose level.

   Developmental NOAEL = 350 mg/kg/day
   Developmental LOAEL = Not observed

This developmental toxicity study is classified acceptable (§83-3[b]) and does satisfy the guideline requirement for a developmental toxicity study in the rabbit.

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C. STUDY DEFICIENCIES - No deficiencies were noted.